J Food Sci Nutr
Vol 15, p 147~151 (2010)
DOI: 10.3746/jfn.2010.15.2.147

J Food Science and Nutrition

Chemical Components of *Atractylodes japonica* Rhizome Oil

– Research Note –

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Abstract

The volatile aroma constituents of *Atractylodes japonica* rhizome were separated by steam distillation extraction method using a Clevenger-type apparatus, and analyzed by gas chromatography-mass spectrometry (GC/MS). The yield of the essential oil from *Atractylodes japonica* was 1.0% (v/w), and its color was pale yellow. Forty-five volatile flavor compounds, which make up 93.86% of the total peak area, were tentatively identified in the rhizome oil. The oil contained 32 hydrocarbons (79.19%) with sesquiterpene hydrocarbon predominating, 3 esters (12.46%), 4 alcohols (0.11%), 1 ketone (0.01%), 2 aldehydes (0.02%), and 3 miscellaneous compounds (2.07%).

Key words: *Atractylodes japonica*, aromatic medicinal plant, steam distillation, GC/MS

INTRODUCTION

Atractylodes japonica, one of the well known varieties within the *Atractylodes* genus, belongs to the family *Asteraceae*, a perennial, aromatic, and medicinal plant (1,2). The *Asteraceae* family comprises approximately one thousand genera and thirty thousand species, distributed more or less around the globe (3,4). There are numerous edible aromatic and medicinal herbaceous plants growing wild in Europe and Asia. Some of them are known for their functional, biological, and physio chemical properties. Aromatic medicinal plants have considerable importance because of their historical applications in folk medicine, and their current potential for commercial value in fields such as food additives and enhancers, cosmetics, and pharmaceuticals (5,6). *Atractylodes japonica* has been reported to have bone marrow cell proliferation activity through the intestinal immune system (7-9). The biological properties of terpenoid rich essential oils from aromatic plants include inhibitory action against microorganisms through membrane disruption (10,11). With the growing interest in the use of essential oils from medicinal plants in both the food and the pharmaceutical industry, a systematic examination of the volatile flavor constituents of *Atractylodes japonica* will be highly useful. In this study, the modified method of simultaneous steam distillation extraction (SDE) was employed, which eliminates the use of organic solvents that might contaminate the plant's distillates.

MATERIALS AND METHODS

Plant materials

Atractylodes japonica was purchased at Gyungdong Herbal Market (Seoul, Korea) in April of 2007. This plant had been harvested in October of 2006 from Uiseong (Gyeongsangbuk-do) province in western Korea. The sample was kept at -70°C in airtight bags until analysis was carried out.

Extraction of essential oil

One kg of dried *Atractylodes japonica* rhizome was crushed for 30 sec by the laboratory scale grinder (NJ-8060SM, NUC Electronics, Seoul, Korea), and extracted by steam distillation extraction method for 3 hr by a Clevenger-type apparatus (Hanil Lab Tech Ltd., Incheon, Korea) (12). The essential oil obtained was dried over anhydrous sodium sulfate overnight, measured, and stored in hermetically sealed dark-glass containers in a freezer at -4°C until it was tested and analyzed by GC/MS.

Gas chromatography-mass spectrometry (GC-MS)

An Agilent 6890 gas chromatography/5973 mass selective detector (Agilent Co., Palo Alto, CA, USA) was employed. Analysis was carried out on an HP-5MS (5%-phenyl-methylpolysiloxane) capillary column (30 m length × 0.25 mm i.d. × 0.25 μm film thickness; Agilent Co., Palo Alto, CA, USA) using a micro syringe. Helium gas was used as carrier gas at a flow rate of 1.0 mL/min. The oven temperature was maintained at 40°C for 5 min and then programmed to increase as follows: from 40 to 150°C at a rate of 3°C/min and holding at 150°C for 5 min, and then 150 to 220°C at a rate of 7°C/min and holding at 220°C for 5 min. The temperatures of the injector and detector were 250 and 280°C, respectively. The sample 0.1 μL, previously dissolved in methylene chloride, was injected in split mode with a split ratio of 10:1. The MS condition were: ionization energy of

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the mass selective detector was 70 eV, scanning interval 0.5 sec and detector voltage 1.2 kV, and the mass scanning ranges were recorded at m/z 33～330.

Identification of chemical compounds

The components of the steam-distilled oil from *Atractylodes japonica* were tentatively characterized by means of comparison of their RIs on an HP-5MS capillary column, which were determined relative to the retention time of a homologous series of *n*-alkanes with linear interpolation with those of authentic compounds. The constituents were also identified by comparison of their RIs with those of other essential oils which had been identified earlier (13). Identification was also achieved by comparison with the fragmentation pattern of the authentic compounds from the mass spectra with those in an on-line computer library (Wiley 275) (Agilent Co.). These measurements were confirmed by matching the observed mass spectra with those of reference compounds in the data system. The RIs of the compounds, determined using *n*-alkanes [Alkane Standard Solution (04070, 04071), (C_8～C_{20}, C_{21-40}), Standard for GC, Fluka, Buchs, Switzerland] as external references, were compared with the published data (14,15). Several compounds were identified with those of the literature (16-18), and identification based on co-injection with authentic compounds (Acros, Sigma-Aldrich, St. Louis, MO, USA). The relative amount of individual components from the oil are expressed as peak area % relative to total peak area from the based on the ratio of the peaks obtained from the mass total ion chromatogram, and also marked quality percentage of the volatile flavor compounds from the GC/MS data.

RESULTS AND DISCUSSION

Profiles of volatile aroma components

There are various extraction methods of plant essential oils: simultaneous steam distillation extraction (SDE), hydro-distillation extraction (HDE), steam distillation extraction, and head space method. SDE is a useful method for extraction of the many volatile flavor components, however it has several limitations, such as using an organic solvent, boiling off-flavor and long experimental time. Recently, the head space solid phase micro-extraction (HS-SPME) method has become the favored method for the analysis of plant aroma; however it has limitations in cell experiments and bioactivity testing. In this study, the modified method of SDE was employed. The yield of the essential oil from 1 kg plant material of *Atractylodes japonica* rhizome was 1.0% (v/w), and the color was pale yellow. The list of detected compounds in the steam distilled oil of *Atractylodes japonica*, along with their retention time, relative percentage, retention indices, and percentage amounts of compound classes are given in Table 1. The data are mean values of triplicates. As shown in Table 1, 45 volatile flavor compounds, which make up 93.86% of the total composition, were tentatively characterized in the essential oil of *Atractylodes japonica*. They consist of 32 hydrocarbons compounds with sesquiterpene predominating, 4 alcohols, 3 esters, 2 aldehydes, 1 ketone, and 3 miscellaneous compounds. Furanodiene, which takes up 26.41% of the total peak area, was the predominant compound. 2,7-dimethoxy-3,6-dimethylnaphthalene (13.56 %), 6,7-dihydroxyxanthotoxin (12.30%), valenecene (7.56 %), and germacrene B (6.28%) were the next most abundant compounds of *Atractylodes japonica* rhizome oil. β-Selinene, β-caryophyllene, isocomene, 9,10-dehydroisolongifolene, β-elemene, and caryophyllene oxide were the compounds with concentrations higher than 2% as % peak area. These were followed by β-sesquiphellandrene, α-humulene, β-patchoulene, β-maaliene, and aristolene (>1%). The major functional group was terpene hydrocarbon with sesquiterpene predominating. The main volatile aroma compounds with concentrations higher than 10% as % peak area were furanodiene, 2,7-dimethoxy-3,6-dimethylnaphthalene, and 6,7-dihydroxyxanthotoxin.

Hydrocarbons

Thirty-two hydrocarbons, which make up 79.19% were tentatively characterized in the essential oil of *Atractylodes japonica*. Eleven monoterpenes [α-pinene, β-pinene, myrcene, α-phellandrene, δ-3-carene, α-terpinene, *p*-cymene, β-phellandrene, (*E*)-β-ocimene, γ-terpinene, and α-terpinolene] accounted for 1.04% of the total peak area. Monoterpenic compounds can be derived from the condensation of two isoprene units. The *p*-menthane skeleton appears to represent the most stable monoterpene structure. Six dines having the *p*-menthane skeleton occur in nature, these being limonene, α-terpinene, α-phellandrene, β-phellandrene, terpinolene, and γ-terpinene (19). Among them, four compounds (α-phellandrene, α-terpinene, γ-terpinene, and β-phellandrene) were identified in the essential oil of *Atractylodes japonica*. γ-Terpinene has a slightly bitter-herbaceous flavor at high concentration, but a pleasant citrus flavor at low concentration, and has been used in the reconstruction of certain essential oils, mainly for flavor purposes (20). Eighteen sesquiterpenes [β-maaliene, aristolene, β-patchoulene, isocomene, *allo*-aromadendrene, β-elemene, γ-elemene, (-)-dehydroaromadendrene, β-caryophyllene, α-humulene, β-selinene, aromadendrene, β-sesquiphellandrene, β-guaiene,

Table 1. The volatile flavor compounds of *Atractylodes japonica* rhizome

Compounds	RT[1)]	RI[2)]	QA%[3)]	PA%[4)]	Method of ID[5)]
α-Pinene	09.91	0940	95	0.27	RT, MS/RI
β-Pinene	11.96	0970	96	0.03	RT, MS/RI[6)]
Myrcene	13.01	0985	98	0.03	RT, MS/RI
α-Phellandrene	13.50	1001	93	0.34	RT, MS/RI
δ-3-Carene	13.76	1005	97	0.08	RT, MS
α-Terpinene	14.13	1017	94	tr	RT, MS/RI[7)]
p-Cymene	14.55	1024	94	0.07	RT, MS/RI
β-Phellandrene	14.72	1029	81	0.12	RT, MS/RI
1,8-Cineol	14.81	1031	98	tr	RT, MS/RI
(E)-β-Ocimene	15.44	1043	95	0.06	RT, MS/RI
γ-Terpinene	16.32	1062	97	tr	RT, MS/RI[*]
2-Undecene	17.48	1088	87	tr	RT, MS/RI[7)]
α-Terpinolene	17.78	1095	98	0.04	RT, MS
Nonanal	18.78	1113	79	0.01	RT, MS
p-Menth-2-en-1-ol	20.33	1138	90	tr	RT, MS
Camphor	20.48	1143	81	0.01	RT, MS
Citronellal	21.16	1154	98	0.01	RT, MS/RI
Terpinen-4-ol	22.36	1172	83	0.03	RT, MS/RI[*]
p-Cymen-8-ol	22.62	1177	79	0.02	RT, MS
Methyl salicylate	22.96	1183	97	0.01	RT, MS
Estragol	24.10	1207	93	0.02	RT, MS/RI[*]
Bornyl acetate	27.25	1280	90	0.15	RT, MS/RI
Aristolene	29.23	1328	95	1.23	RT, MS
β-Maaliene	29.72	1356	87	1.34	RT, MS
β-Patchoulene	31.35	1406	97	1.80	RT, MS/RI
Isocomene	31.70	1411	97	2.15	RT, MS/RI
allo-Aromadendrene	31.84	1414	79	0.20	RT, MS
β-Elemene	31.99	1416	94	2.22	RT, MS/RI
Methyl eugenol	32.68	1428	99	0.06	RT, MS/RI
β-Caryophyllene	33.16	1436	97	3.26	RT, MS/RI
γ-Elemene	33.70	1445	99	0.76	RT, MS/RI
α-Humulene	34.77	1460	98	1.45	RT, MS
β-Selinene	35.98	1485	96	4.20	RT, MS/RI
(-)-Dehydroaromadendrene	36.18	1490	98	0.57	RT, MS
Aromadendrene	36.63	1497	99	0.26	RT, MS
β-Guaiene	37.06	1501	99	0.58	RT, MS
β-Sesquiphellandrene	37.47	1512	93	1.65	RT, MS
Valenecene	38.20	1530	97	7.56	RT, MS/RI
Germacrene B	38.95	1548	98	6.28	RT, MS/RI
9,10-dehydro-isolongifolene	39.60	1565	86	2.67	RT, MS
Caryophyllene oxide	40.04	1576	99	2.04	RT, MS/RI
Furanodiene	43.03	1640	95	26.41	RT, MS/RI[8)]
2,7-Dimethoxy-3,6-dimethylnaphthalene	43.50	1650	98	13.56	RT, MS
6,7-Dihydroxyxanthotoxin	46.89	1722	77	12.30	RT, MS
14 β-Pregnane	56.96	2201	82	tr	RT, MS

[1)]RT is retention time. [2)]RI: Retention indices were determined by using *n*-alkanes ($C_8 \sim C_{22}$) as external references. [3)]QA% means quality% of the MS data (n=3). From *Atractylodes japonica* rhizome oil. [4)]PA% means peak area %, average (n=3) of the relative percentage of the peak area in the MS total ion chromatogram. tr, trace; mean value $<0.01\%$. [5)]Method of identification based on reference no. 14, 15. MS, mass spectrum was consistent with that of Wiley mass spectrum database (2001, Hewlett Packard Co., Palo Alto, CA, USA). RI was consistent with that of the literature. [6)]Identification based on reference no. 16. [7)]Identification based on reference no. 17. [8)]Identification based on reference no. 18. [*]Identification based on co-injection with authentic compounds (Acros, Sigma-Aldrich, St. Louis, MO, USA).

valenecene, germacrene B, 9,10-dehydro-isolongifolene, and furanodiene (64.59%)] from *Atractylodes japonica* steam distilled oil were investigated by GC/MS. Among them, furanodiene was the predominant sesquiterpene hydrocarbon compound with a concentration of 26.41%. Recently, furanodiene was also characterized from the three species of *Curcuma* rhizome extracts (21) and demonstrated several bioactivities (22,23). The volatile flavor compound 2,7-dimethoxy-3,6-dimethylnaphthalene was investigated as the main compound of the essential oil from *Atractylodes macrocephala* rhizome (24). Additionally, this compound was detected in the *Pycanthus angolensis* (Welw) Ward (Myristicaceae) that grows throughout west and central Africa, and its extracts revealed significant antimicrobial activity (25). We also identified α-humulene in this study, with a concentration of 1.45%. Recently, it was reported that α-humulene of *Cordia verbenacea* oils was able to diminish edema formation and has anti-cancer effects (26). This compound is also the major volatile component of essential oils from *Myristica malabarica* L. and *Gymnacranthera canarica* (King) Warb. (27). The major hydrocarbons with concentrations higher than 6 % as % peak area were furanodiene, 2,7-dimethoxy-3,6-dimethylnaphthalene, valenecene, and germacrene B.

Alcohols and aldehydes

Various alcohol compounds were detected in this sample using our analytical methodology, although most of the alcohol compounds were found in very small amounts. There were four alcohol compounds that make up 0.11% in *Atractylodes japonica* rhizome oil with terpinen-4-ol, *p*-menth-2-en-1-ol, *p*-cymen-8-ol, and methyl eugenol. Among them, terpinen-4-ol, also called as 4-terpineol, is volatile flavor compound that has recently been used for aroma therapy with lavender oil. Additionally, it is known to be one of the major volatile flavors of the tea tree oil. Indeed, the quality of the tea tree oil depends on the concentration of this compound (28). Aldehydes are intermediates between the alcohols and the acids. The aldehydes have a lower molecular weight and are characterized by their unpleasant and pungent odors and irritating effect on the nose. As their molecular weight increases, the odor profile of the aldehydes gradually leads a more pleasant, fruity character. This change is especially noticeable for aldehydes C_8 to C_{10}, which have very attractive floral odor (19). Two aldehydes, including nonal and citronellal, were detected in the essential oil of *Atractylodes japonica*, accounting for 0.02%.

Ketones and esters

Camphor was characterized in small amount (0.01%) of *Atractylodes japonica* distilled oil. The ketones give a wide range of aromatic effects, and most of them are pleasing. Those of higher molecular weight have a marked floral character (20). Camphor is a saturated ketone having camphene skeleton, and is obtained industrially via pinene, camphene, and isoborneol (19). Three esters constituted 12.46% of the *Atractylodes japonica* rhizome distillate, which includes 6,7-dihydroxyxanthotoxin, bornyl acetate, and methyl salicylate. Among those, 6,7-dihydroxyxanthotoxin is the cyclic ester compound. Acetate is of prime importance in the formulation of imitation flavors (20).

In this study we determined the constituents of *Atractylodes japonica* rhizome essential oil by GC/MS. The common characteristics of the steam distilled oil from *Atractylodes japonica* produced in Korea and identified in this work were its high content of terpene hydrocarbons, with sesquiterpenes predominating, and its low contents of alcohol, aldehyde, and ketone. The compounds identified consist of 32 hydrocarbons compounds, 4 alcohols, 3 esters, 2 aldehydes, 1 ketone, and 3 miscellaneous compounds. Furanodiene was the predominant compound, encompassing 26.41% of the total peak area, followed by 2,7-dimethoxy-3,6-dimethylnaphthalene, 6,7-dihydroxyxanthotoxin, valenecene, and germacrene B, which had concentrations higher than 6% as % peak area of *Atractylodes japonica* rhizome oil. β-Selinene, β-caryophyllene, 9,10-dehydro-isolongifolene, β-elemene, isocomene, caryophyllene oxide, β-patchoulene, β-sesquiphellandrene, α-humulene, β-maaliene, and aristolene, were the compounds with concentrations higher than 1% as % peak area of the total composition. The volatile chemical compounds furanodiene and 2,7-dimethoxy-3,6-dimethylnaphthalene have been reported to have several bioactivities and anti-cancer effects. We envision a possible use of *Atractylodes japonica* rhizome oil in the food and pharmaceutical industries because of the bio-functional properties of its two most abundant compounds.

ACKNOWLEDGEMENT

This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0094018).

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(Received February 1, 2010; Accepted March 17, 2010)